Form PTO-1390 (REV 10-2000) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
1581.0780000/RWE/CEJ	
U.S APPLICATION NO. (IF KNOWN, SEE 37 C.F R. § 1.5) To be assigned 09/763750	
INTERNATIONAL APPLICATION NO PCT/GB99/02828	INTERNATIONAL FILING DATE 27 August 1999
PRIORITY DATE CLAIMED 27 August 1998	

O I P E JC47 FEB 2 7 2001 PATENT & TRADEMARK OFFICE

TITLE OF INVENTION
Superoxide Dismutase as a Vaccine Antigen

APPLICANT(S) FOR DO/EO/US
GORRINGE, Andrew Richard; KROLL, John Simon; LANGFORD, Paul Richard; and ROBINSON, Andrew

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)).
4. ☐ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 372(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information: A copy of The International Preliminary Examination Report with annexes; 37 C.F.R. § 1.136(a)(3) Authorization to Treat a Reply As Incorporating An Extension of Time *(in duplicate)*; and Two (2) return postcards.

U.S. APPLICATION NO (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
To be assigned 09/763750	PCT/GB99/02828	1581.0780000/RWE/CEJ

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1000.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$710.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) ..... **$ 100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00	

Claims	Number Filed		Number Extra	Rate		
Total Claims	27 - 20	=	7	X **$18.00**	$ 126.00	
Independent Claims	10 - 3	=	7	X **$80.00**	$ 560.00	
Multiple dependent claim(s) (if applicable)				+ **$270.00**	$ 0.00	
TOTAL OF ABOVE CALCULATIONS =					$ 1,676.00	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.					$0.00	
SUBTOTAL =					$ 0.00	
Processing fee of $130.00 for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +					$ 0.00	
TOTAL NATIONAL FEE =					$ 1,676.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +					$ 0.00	
TOTAL FEES ENCLOSED =					$ 1,676.00	
					Amount to be refunded:	$
					charged:	$

a. ☒ A check in the amount of $1,676.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 19-0036. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit Under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:
STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
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NAME

32,893
REGISTRATION NUMBER

JC02 Rec'd PCT/PTO 27 FEB 2001
09/763750

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	
Gorringe *et al.*	Art Unit: To Be Assigned
U.S. National Phase of PCT/GB99/02828	Examiner: To Be Assigned
Filed: (International Filing Date: August 27, 1999)	Atty. Docket: 1581.0780000/RWE/CEJ
For: **Superoxide Dismutase as a Vaccine Antigen**	

Preliminary Amendment

Commissioner for Patents
Washington, D.C. 20231

Sir:

In advance of prosecution, please amend the above-captioned application as follows.

Amendments

In the Claims:

Please amend the claims as follows:

Please cancel claims 21-27 without prejudice to or disclaimer of the subject matter contained therein.

3. (Once amended) A pharmaceutical composition according to Claim 1 [or 2], wherein said composition provides protective immunity to *Actinobacillus pleuropneumoniae* infection.

4. (Once amended) A pharmaceutical composition according to Claim[s] 1 [or 2], wherein said composition provides protective immunity to infection from a gram negative bacterial species selected from the group consisting of *Pasteurellaceae; Neisseria; Haemophilus; Salmonella; and Escherichia.*

5. (Once amended) A pharmaceutical composition according to [any previous] claim 1, wherein the Cu,Zn-SOD is obtainable from a recombinant gene cloned from bacteria.

8. (Once amended) A vaccine according to Claim[s] 6 [or 7], wherein said vaccine provides protection against meningococcal infection.

9. A method of preparing a pharmaceutical composition comprising:-

1) isolating a gene for a bacterial Cu,Zn-SOD of the dimeric type or a fragment, variant or derivative of the Cu,Zn-SOD, wherein antibodies raised against said fragment, variant or derivative also bind the full length intact Cu,Zn-SOD; and

2) (a) synthesizing the Cu,Zn-SOD or fragment, variant or derivative from the gene; and combining said Cu,Zn-SOD, fragment, variant or derivative, with a pharmaceutically acceptable carrier, or

(b) combining said gene with a pharmaceutically acceptable carrier.

13. (Once amended) A pharmaceutical preparation according to Claim 10 [or 11], wherein said composition provides protective immunity to infection from a gram negative bacterial species selected from the group consisting of *Pasteurellaceae; Neisseria; Haemophilus; Salmonella; and Escherichia.*

14. (Once amended) A pharmaceutical preparation according to [any of] Claim[s] 10 [to 13], wherein said antibody displays bactericidal activity.

15. (Once amended) A multivalent vaccine comprising a plurality of Cu,Zn-SODs of the dimeric type, or fragments, derivatives or variants thereof, wherein antibodies raised against said fragments, derivatives or variants also bind full length Cu,Zn-SOD, and wherein said plurality of Cu,Zn-SOD[S]s are from the same or different species of Gram negative bacteria.

16. (Once amended) A multivalent vaccine comprising a bacterial Cu,Zn-SOD of the dimeric type, or fragments, derivatives or variants [thereof] wherein antibodies

raised against said fragments, derivatives or variants also bind [intact] full length Cu,Zn-SOD[;], and a second protein that is not a Cu,Zn-SOD.

17. (Once amended) A multivalent vaccine according to Claim[s] 15 [or 16], wherein said vaccine provides protective immunity to meningococcal disease.

18. (Once amended) [Use of] A method of treating an individual with a bacterial infection comprising administering a composition comprising a bacterial Cu,Zn-superoxide dismutase of the dimeric type, or a fragment, derivative or variant of the Cu,Zn-SOD, wherein antibodies raised against said fragment, variant or derivative also bind intact full length Cu,Zn-SOD [;in the manufacture of a medicament for treatment or prevention of bacterial infection].

19. (Once amended) [Use] A method according to Claim 18, wherein the bacterial infection is due to Gram negative species of bacteria.

20. (Once amended) [Use] A method according to Claim 18 [or 19], wherein the bacterial infection is due to meningococcal infection.

Please add the following claims:

--28. A method of treating an individual with a bacterial infection comprising administering a composition comprising an effective amount of an antibody specific to bacterial Cu,Zn-SOD of the dimeric type, or a fragment of said antibody.

29. A method according to Claim 28 wherein the antibody is a monoclonal antibody.

30. A method of treating an individual with a bacterial infection comprising administering a composition comprising a nucleic acid encoding a bacterial Cu,Zn-superoxide dismutase of the dimeric type, or a fragment, derivative or variant of the Cu,Zn-SOD, wherein antibodies raised against said fragment, variant or derivative also bind intact full length Cu,Zn-SOD.

31. A method of treating or preventing bacterial infection comprising administering an effective amount of a bacterial Cu,Zn-SOD or fragment, variant or derivative of the Cu,Zn-SOD, wherein antibodies raised against said fragment, variant or derivative also bind intact full length Cu,Zn-SOD.

32. A method according to Claim 18, wherein said composition provides protective immunity to *Actinobacillus pleuropneumoniae* infection.

33. A method according to Claim 19, wherein said gram negative bacterial species are selected from the group consisting of *Pasteurellaceae; Neisseria; Haemophilus; Salmonella; and Escherichia.*

34. A method according to Claim 28, wherein the bacterial infection is a meningococcal infection.--

Remarks

I. Support for the Amendments

Support for the foregoing amendments is replete throughout the specification. The amendments to claims 3, 4, 5, 8, 13, 14, and 17 were made merely to change the claim dependencies. The amendment to claim 15 was made to correct an obvious typographical error. Amendments to claims 18-20 were made merely to put the claims in proper format for examination in the United States. Support for the amendments to claim 18 can be found in the specification from page 7, line 24, through page 8, line13; and page 5, lines 11-27. Support for new claims 28-34 can be found in the specification at page 3, lines 21-29; page 5, lines 11-27, page 6, lines 2-11; page 8, lines 5-13; page 8, line 30, through page 9, line 7; and in Example 1, page 9, line 16, through page 13, line 19. Accordingly, the foregoing amendments are fully supported in the specification and claims as originally filed, and therefore do not add new matter. Their entry and consideration are therefore respectfully requested.

II. Status of the Claims

By the foregoing amendments, claims 3-5, 8, 9 and 13-20 have been amended, claims 21-27 have been cancelled and new claims 28-34 have been added. Upon entry of the foregoing amendments claims 1-20 and 28-34 are pending in the present application, with claims 1, 6, 9, 10, 15, 16, 18, 28, 30, and 31 being the independent claims. These changes are believed to introduce no new matter, and their entry is respectfully requested.

VII. Summary

If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Robert W. Esmond

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Date: Feb 27, 2001

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FEB 28 2001 09:52

04 JUN 2001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: GORRINGE *et al.*	Art Unit: To be assigned
Appl. No. (U.S. National Phase PCT/GB99/02828; U.S. Appl. No. 09/763,750)	Examiner: To be assigned
International Filing Date: August 27, 1999	Atty. Docket: 1581.0780000/RWE
For: **Superoxide Dismutase as a Vaccine Antigen**	

Second Preliminary Amendment and Submission of Sequence Listing

Commissioner for Patents
Box PCT
Washington, D.C. 20231

Sir:

In response to the Notification to Comply With Requirements For Patent Applications, dated April 4, 2001, in the above identified matter, and in advance of prosecution, please amend the application as follows:

In the Specification:

Please insert the Sequence Listing at the end of the application.

Remarks

No new matter has been added. The specification has been amended to direct the entry of the Sequence Listing after the claims of the above-identified application.

In accordance with 37 C.F.R. § 1.821(g), this submission includes no new matter.

In accordance with 37 C.F.R. § 1.821(f), the paper copy of the Sequence Listing and the computer readable copy of the Sequence Listing submitted herewith in the above application are the same.

It is respectfully believed that this application is now in condition for examination. Early notice to this effect is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Robert W. Esmond

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Date: June 4, 2001

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SKGF Rev 1 1/26/01 mac

FILE - 09763750 - 06/05/2001

SEQUENCE LISTING

<110> Gorringe, Andrew Richard
Langford, Paul Richard
Kroll, John Simon
Robinson, Andrew

<120> SUPEROXIDE DISMUTASE AS A VACCINE ANTIGEN

<130> 1581.0780000

<140> US 09/763,750
<141> 2001-02-27

<150> PCT/GB99/02828
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09/763750
JC02 Rec'd PCT/PTO 27 FEB 2001

SUPEROXIDE DISMUTASE AS A VACCINE ANTIGEN

The present invention relates to pharmaceutical compositions for treating and/or vaccinating against bacterial infection and to methods of manufacturing such compositions. In particular, the invention relates to pharmaceutical compositions which comprise superoxide dismutase or antibodies thereto.

At present most bacterial infections in humans or animals are treated after infection has set in by administration of antibiotic drugs. As many more strains of pathogenic bacteria become resistant to current antibiotics, the range of options open for treatment decreases. Moreover, many antibiotics can cause dangerous side effects upon individuals or animals taking them, for example allergy to penicillin or the toxicity of sulpha drugs. Furthermore, antibiotic treatment is sometimes only effective if the drug is taken regularly over a period of time thus maintaining a constant level of therapeutic agent in circulation. If individuals forget or are unable to maintain the course of antibiotic treatment then it may be rendered ineffective.

Some bacterial infections progress very quickly, sometimes too quickly for antibiotic treatment to have much effect unless administered at a very early stage in the course of the infection. Meningococcal disease is one example of such a virulent infection and is caused by the pathogen *Neisseria meningitidis*. In many cases symptoms of disease at first resemble those of influenza and thus infected individuals often delay in seeking medical attention. Vaccines based on polysaccharides present on the surface of some *N. meningitidis* serogroups are available at present but they show limited protection against infection. Moreover, the surface polysaccharide of serogroup B strains of *N. meningitidis* (causative agent of over half the cases of meningococcal disease in the UK) are only weakly immunogenic and are not included in current vaccines.

Accordingly, it is an object of the invention to provide a pharmaceutical composition comprising a vaccine antigen or an antibody that effectively protects against or ameliorates bacterial infection. It is a further object of the invention to provide a pharmaceutical composition comprising a vaccine antigen that protects against meningococcal disease. It is yet a further object to provide a vaccine antigen that also provides protective immunity against a broader range of infectious bacteria. It is a further object to provide a method of manufacturing antibodies that can provide protective immunity to a range of bacterial pathogens when included in a pharmaceutical preparation. It is still a further object of the invention to provide a multivalent vaccine which provides protective immunity to a wide range of bacterial infections.

Cu,Zn-Superoxide Dismutase (Cu,Zn-SOD) is an metalloenzyme found in many prokaryotic and eukaryotic organisms. It catalyses the reduction of the superoxide radical anion, O_2^-, to hydrogen peroxide and molecular oxygen, thus playing an important role in the removal of cytotoxic free radicals from the organism. In bacteria Cu,Zn-SODs have been identified in the periplasm of a number of Gram negative species including *N. meningitidis*, *Haemophilus ducreyi*, *Haemophilus parainfluenzae*, *Actinobacillus pleuropneumoniae* and *Pasteurella multocida* (Kroll et al, 1995). The enzyme can exist as a dimer or a monomer, and examples of monomeric Cu,Zn-SODS are those from *Brucella abortus* and *Escherichia coli* (Pesce, et al, 1997). It is believed that Cu,Zn-SOD provides a defence for the bacterium against the burst of oxygen free radicals released by phagocytic host cells, such as macrophages, during infection (Wilks et al, 1998; Farrant et al, 1997).

A known attempt to utilise Cu,Zn-SOD as a vaccine antigen has not been successful. Tabatabai (Tabatabai and Pugh, 1994) showed that a synthetic fragment of a monomeric Cu,Zn-SOD (denoted as peptide 3) from *B. abortus* was able to provide a low level of immunity in mice against *Brucella* infection, but the level of protection provided was lower than that seen when using

Brucella cell surface proteins and lipopolysaccharide antigens. Moreover, vaccination with the entire Cu,Zn-SOD failed to provide protective immunity at all. Tabatabai concluded that the antigenic fragment contained within *Brucella* Cu,Zn-SOD was counteracted by other parts of the protein which prevented it from eliciting an antigenic activity. This masking property was in fact so strong that even a mixture of synthetic peptides that included peptide 3 elicited no protective immunity to *Brucella* infection. Thus the study by Tabatabai et al teaches against the use of either fragments of Cu,Zn-SOD or full length Cu,Zn-SOD as an effective antigen that could provide protective immunity to bacterial infection.

Cu,Zn-SODs from eukaryotes and most Gram negative bacteria form dimers in their native form. However, the Cu,Zn-SODs of *E.coli* and *B. abortus* are atypical in that they are normally monomeric (Pesce et al, 1997). In all Gram negative bacteria that produce Cu,Zn-SOD the protein is localised to the periplasmic space between the outer cell wall and the cell membrane.

The present invention relates to the surprising discovery that a monoclonal antibody raised against a recombinant Cu,Zn-SOD fusion protein from *A. pleuropneumoniae* has bactericidal activity against *N. meningitidis* and protects mice against challenge with *N. meningitidis*. Furthermore, a recombinant, dimeric Cu,Zn-SOD and immunogenic fragments from *A. pleuropneumoniae* (a pig pathogen) act as antigen that can confer protective immunity not only against this organism but also against other Gram negative human pathogens such as *N. meningitidis*, *H. ducreyi*, *H. parainfluenzae* and *P. multocida*. Thus the Cu,Zn-SOD is suitable as a vaccine component against both animal and human bacterial diseases such as meningococcal disease (caused by *N. meningitidis*) or chancroid (caused by *H. ducreyi*), or porcine pleuropneumonia (caused by *A. pleuropneumoniae*).

Accordingly, a first aspect of the present invention provides a composition comprising a Cu,Zn-SOD of the dimeric type, or a fragment, variant or

derivative thereof, and a pharmaceutically acceptable carrier. The first aspect of the invention also provides a composition comprising a nucleic acid encoding a Cu,Zn-SOD of the dimeric type, or a fragment, variant or derivative thereof, and a pharmaceutically acceptable carrier. By "dimeric" we mean a SOD that naturally forms dimers under normal conditions, e.g. is found in dimeric form in nature. A pharmaceutically acceptable carrier could comprise an approved adjuvant such as alum or any other adjuvant approved for pharmaceutical purposes. The Cu,Zn-SOD can be from any bacteria, though especially from known pathogenic bacteria, and from *N. meningitidis* as an example.

The present invention is not to be restricted to the use of full length or wild type Cu,Zn-SOD of the dimeric type. An antigenic fragment of the Cu,Zn-SOD may also be used in a vaccine formulation. The fragment preferably comprises a region of the Cu,Zn-SOD that is on the surface of the protein, although any fragment that confers protective immunity to bacterial infection is suitable; and the term "fragment" is intended to encompass any fragment against which an antibody may be raised which antibody binds intact, full length SOD. Moreover, mutant variants which have been modified to increase antigenicity or fusion protein derivatives between all or a part of a Cu,Zn-SOD and another protein for the purposes of purification or increasing antigenicity may also be suitable for use in pharmaceutical compositions. Vaccine components of the invention also include derivatives and variants of Cu,Zn-SOD. The term "derivative" is intended to encompass combinations of Cu,Zn-SOD with other proteins or molecules, including carbohydrates to form conjugate vaccines, the derivative retaining antigenicity such that an antibody raised against the derivative binds intact, full length SOD. The term "variant" is intended to encompass a polypeptide having an amino acid sequence that varies from that of intact, full length SOD, but such that antibodies raised against the variant bind intact, full length SOD.

In a preferred embodiment of the invention the pharmaceutical composition

provides protection against meningococcal infection and/or disease. In further preferred embodiments of the invention the pharmaceutical composition provides protective immunity to infection from *Actinobacillus* species (e.g. *A. pleuropneumoniae, A. actinomycetemcomitans*), *Pasteurellaceae* species (e.g. *P. multocida*), *Neisseria* species (e.g. *N. meningitidis*), *Haemophilus* species (e.g. *H. influenzae, H. parainfluenzae, H. ducreyi*), *Escherichia coli*, *Salmonella* species and other bacteria producing a Cu,Zn-SOD. In a specific embodiment of the invention the Cu,Zn-SOD is expressed from a recombinant gene cloned from *Actinobacillus pleuropneumoniae*.

It is an advantage of the present invention that a Cu,Zn-SOD of the dimeric type, or a fragment, variant or derivative thereof, can confer protective immunity to infection from a broad range of bacterial pathogens. It is of further advantage that the present invention provides for pharmaceutical compositions comprising Cu,Zn-SODs, or a fragment, variant or derivative thereof, that are protective against bacterial infection in both humans and animals and in particular to meningococcal disease. It is of still further advantage that an antibody to a Cu,Zn-SOD from one species of bacteria can provide protective immunity to infection from a plurality of other species of bacteria, in particular that the invention provides protective immunity to meningococcal disease. A further advantage of the present invention is that Cu,Zn-SOD is relatively abundant and can be easily purified from bacterial cultures. Moreover, recombinant Cu,Zn-SODs can be fused to other proteins, such as glutathione-S-transferases, to facilitate purification from bacterial cultures expressing the fusion protein, and the Cu,Zn-SOD moiety retains both antigenicity and biological activity.

In use of the invention, a Cu,Zn-SOD is cloned from the pig pathogen *Actinobacillus pleuropneumoniae*, to give a recombinant form of the gene. The recombinant Cu,Zn-SOD gene is optionally linked to, such as by fusing, a glutathione-S-transferase gene to enable easy purification of the fusion protein when expressed in bacteria. A pharmaceutical preparation is prepared

comprising the purified Cu,Zn-SOD protein and a pharmaceutically acceptable carrier; in one use the carrier includes the adjuvant alum. The pharmaceutical composition is suitably administered to the individual via any route. The nature or form of the composition may be selected from any conventional pharmaceutical composition including but not limited to tablets, capsules, oral compositions, liquids, compositions for infusion, syrups, solutions, powdered formulations and granular formulations. The pharmaceutical composition, in use, stimulates the individual to produce antibodies against the antigenic Cu,Zn-SOD protein, some of which provide protective immunity to a broad range of pathogens. In particular the pharmaceutical composition provides protective immunity to meningococcal disease.

In a specific example of the invention in use, described in more detail below, a Cu,Zn-SOD gene - the *sodC* gene - is isolated from *N. meningitidis* genomic DNA by standard PCR techniques. The product of the PCR reaction additionally incorporates a His-tag sequence (coding for six histidines at the C terminus of the protein). The isolated *sodC* gene plus His-tag is cloned into an expression vector and then transformed into *E. coli*, where the protein product is expressed. The expressed protein is purified on a nickel charged affinity column, to which the His-tag preferentially binds. The SodC protein is then eluted from the affinity column and is suitably incorporated in the pharmaceutical composition of the invention as described above.

A second aspect of the invention provides a vaccine comprising a Cu,Zn-SOD, or a nucleic acid encoding a Cu,Zn-SOD, of the dimeric type, or a fragment, variant or derivative thereof.

In a specific embodiment of the invention the Cu,Zn-SOD of the dimeric type, or a fragment, variant or derivative thereof, is from a recombinant gene cloned from *Actinobacillus pleuropneumoniae*, though the invention also encompasses use of native proteins. In a further preferred embodiment the vaccine provides protective immunity against meningococcal meningitis.

Compositions and vaccines of the invention comprising a nucleic acid which encodes a Cu,Zn-SOD or fragment or derivative thereof are suitably prepared comprising the coding sequence inside a microparticle according to the methods of WO-A-97/17063, incorporated herein by reference.

A third aspect of the invention provides a method of preparing a pharmaceutical composition that consists of:-

1) cloning a gene for a Cu,Zn-SOD of the dimeric type to obtain a recombinant form of the gene; and

2) (a) synthesising Cu,Zn-SOD from the recombinant gene; and combining said Cu,Zn-SOD with a pharmaceutically acceptable carrier, or

(b) combining said gene with a pharmaceutically acceptable carrier.

A fourth aspect of the invention provides for a composition, especially a pharmaceutical preparation comprising an antibody to a Cu,Zn-SOD of the dimeric type, or a fragment, derivative or variant thereof, and a pharmaceutically acceptable carrier. It is optional, but not essential, that the antibody is a monoclonal antibody.

The present invention thus also provides for an antibody preparation that is raised against a dimeric Cu,Zn-SOD, or a fragment, derivative or variant thereof, from one species of Gram negative bacteria and that confers protective immunity to infection from this bacterium and also to infection from a plurality of other Gram negative bacteria. The antibody can be used in pharmaceutical preparations that confer passive immunity to bacterial infection upon a host organism. In the example described in more detail below a monoclonal antibody raised against Cu,Zn-SOD from *A. pleuropneumoniae* provides protection against *N. meningitidis* infection. Thus

the monoclonal antibody is suitable for use in treating acute cases of meningococcal disease as well as in providing passive immunity to future meningococcal disease.

In a specific embodiment of the invention the antibody provides protective immunity to meningococcal disease. Thus it is suitable for use in preparations that provide passive immunity to infection and also for treatment of individuals already suffering from meningococcal infection.

In further specific embodiments of the invention the antibody provides protective immunity to bacterial infection from a range of bacterial pathogens including *Actinobacillus pleuropneumoniae*, *Pasteurellaceae* species, *Neisseria* species and *Haemophilus* species.

In a further preferred embodiment of the invention the antibody displays bactericidal activity. This activity may be directly attributed to the antibody itself or mediated via the complement system.

A fifth aspect of the invention provides for a multivalent vaccine comprising a plurality of Cu,Zn-SODs of the dimeric type, or fragments, derivatives or variants thereof, wherein said plurality of Cu,Zn-SODs are from the same or different species of Gram negative bacteria. A specific embodiment of the invention provides a multivalent vaccine comprising a plurality of Cu,Zn-SODs of the dimeric type, or fragments, derivatives or variants thereof, and one or a number of different bacterial proteins, or a fragment, derivative or variant thereof, that is not or are not a Cu,Zn-SOD. A further preferred embodiment of the invention is a multivalent vaccine that provides protective immunity to meningococcal infection.

A sixth aspect of the invention provides for use of a Cu,Zn-superoxide dismutase of the dimeric type, or a fragment, derivative or variant thereof, in the manufacture of a medicament, e.g. a vaccine, for use against bacterial

infection. In a preferred embodiment of the invention the use is in response to a bacterial infection due to Gram negative species of bacteria. In a specific embodiment of the invention the use is in response to a meningococcal infection. This aspect also provides a method of treating bacterial, particularly meningococcal, infection by administering an effective amount of a Cu,Zn-SOD or a fragment, variant or derivative thereof, or other composition according to the invention.

A seventh aspect of the invention provides for an antibody specific to bacterial Cu,Zn-SOD of the dimeric type, or a fragment, derivative or variant thereof. A preferred embodiment of the invention provides for a monoclonal antibody (MAb) that is specific to bacterial Cu,Zn-SOD.

There now follow examples of specific embodiments of the invention.

Example 1

Cu,Zn-SOD Monoclonal Antibody

Monoclonal antibodies (MAbs) were raised in mice against a glutathione-S-transferase fusion protein with the Cu,Zn-SOD from *Actinobacillus pleuropneumoniae*. The whole fusion protein as expressed in *E. coli* was found to be enzymically active. Of 72 potential hybridomas screened by western blotting, two recognised only *A. pleuropneumoniae* Cu,Zn-SOD, but one also recognised Cu,Zn-SOD *from H. parainfluenzae, H. ducreyi, N. meningitidis* and *A. actinomycetemcomitans*. The latter MAb was used for the passive protection studies.

Passive Protection

Three experiments were performed and all demonstrated that the MAb protects mice against lethal infections with *N. meningitidis.* Adult NIH mice were given an intra-peritoneal (ip) injection with antibody (50μl) per mouse 2h before challenge with *Neisseria meningitidis*. Mice were given further ip

injections of antibody 2,5 and 24h after the challenge. The *N. meningitidis* challenge dose was the stated number of viable bacteria (see tables of results), given as an 0.5ml ip injection, containing 2mg iron dextran. At 24h mice were given a further 2mg iron dextran in an 0.2ml ip dose. Mice were then observed for 72h and the health of the animals noted. The results of these experiments are shown in the following tables.

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EXPERIMENT 1				
VACCINE	CHALLENGE DOSE	SURVIVORS / CHALLENGED		
		DAY 1	DAY 2	DAY 3
growth medium	10^5	5/5	4/5	4/5
growth medium	10^6	5/5	3/5	3/5
50μl MAb	10^5	5/5	5/5	5/5
50μl MAb	10^6	5/5	5/5	5/5
5μl MAb	10^5	5/5	5/5	5/5
5μl MAb	10^6	5/5	4/5	4/5
Polyclonal serum *	10^5	5/5	5/5	5/5
Polyclonal serum *	10^6	5/5	5/5	5/5

* - raised against meningococcal outer membrane vesicles.

EXPERIMENT 2				
VACCINE	CHALLENGE DOSE	SURVIVORS/CHALLENGED		
		DAY 1	DAY 2	DAY 3
Growth medium	2×10^5	5/5	5/5	5/5
Growth medium	2×10^6	5/5	1/5	0/5
50μl MAb	2×10^5	5/5	5/5	5/5
50μl MAb	2×10^6	5/5	5/5	5/5
5μl MAb	2×10^5	5/5	5/5	5/5
5μl MAb	2×10^6	5/5	3/5	3/5
Polyclonal serum *	2×10^5	5/5	5/5	5/5
Polyclonal serum *	2×10^6	5/5	5/5	5/5

* - raised against meningococcal outer membrane vesicles.

EXPERIMENT 3					
VACCINE	CHALLENGE DOSE	SURVIVORS / CHALLENGED			
		DAY 1	DAY 2	DAY 3	DAY 4
Growth medium	1×10^7	5/5	0/5	0/5	0/5
50μl MAb	1×10^7	5/5	4/5	3/5	2/5
5μl MAb	1×10^7	5/5	1/5	0/5	0/5
Polyclonal serum	1×10^7	5/5	1/5	0/5	0/5
50μl Yersinia MAb	1×10^7	5/5	1/5	0/5	0/5
5μl Yersinia MAb	1×10^7	5/5	0/5	0/5	0/5

Bactericidal Activity

In addition to passive protective activity, the Cu,Zn-SOD MAb has been found to be bactericidal to two strains of meningococcus, with titres of 64-128 compared to a titre of 256 for a Group B polysaccharide specific MAb.

Example 2

Cloning and Expression of Cu,Zn-SOD - Purification of protein via a nickel affinity column

Genomic DNA isolated from *N.meningitidis* strain MC58 was used as a template to amplify the *sodC* gene (Cu,Zn-SOD). Primers were designed using the information in the Genbank database. The 5' primer (SEQ ID NO.1)

incorporates an *Nde*I site at the level of the ATG start codon, allowing the PCR product to be cloned into a variety of pET (Novagen) and pMTL (CAMR) vectors for expression. The 3' primer (SEQ ID NO.2) generates a polyhistidine (6 x His) tag in the translated protein to facilitate nickel affinity purification. The primer sequences are as follows:

"SEQ ID NO.1" : 5' primer: 5' GGC ATA TGA ATA TGA AAA CCT TAT TAG 3'

"SEQ ID NO.2" : 3' primer: 5' GGG CTG AGC TTA TTA GTG GTG GTG GTG GTG GTG TTT AAT CAC GCC ACA TGC CAT ACG TG 3'

The products were sub-cloned into pET22b and transferred to BL21 DE3 for initial expression. His-tagged protein was purified on a nickel charged Hi-Trap column.

Example 3

Purification of protein via a glutathione affinity column

A glutathione-S-transferase (GST) fusion was generated by cloning the *sodC* gene of Example 2 (described above) into one of the pGEX series of vectors (Pharmacia). An N-terminal fusion was generated by cloning a *sodC* PCR product as an *Eco*RI-*Xho*I fragment in frame into pGEX-4T-1 (restriction sites generated by PCR primers containing these sites in-frame immediately upstream or downstream of the *sodC* coding sequence, including 3' stop codon). The expressed protein was a GST-SodC fusion linked by a thrombin cleavage site. The recombinant protein was purified on a glutathione affinity column and cleaved with thrombin to release SodC. This SodC protein has an additional five amino acids on the N terminus, compared to wild type protein (sequence GSPQF).

Example 4

Purification of protein via a glutathione affinity column - Alternative method

This alternative cloning strategy varies from the method of Example 3 in that it allows reduction in the number of additional N terminal amino acids on the cleaved SodC product. This is achieved by removing the internal MC58 *sodC* *Bam*HI restriction site by site directed mutagenesis. PCR amplification of this altered gene with a 5' *Bam*HI site in-frame upstream of the ATG start codon facilitates cloning of a *Bam*HI-*Eco*RI fragment into pGEX-2T vector (Pharmacia). The resultant GST-SodC fusion protein is expressed and SodC released by cleavage with thrombin protease, giving rise to SodC product with just a two amino acid N terminal 'tag' (sequence GS).

References:

Farrant, J.L. et al. (1997); Bacterial copper- and zinc- cofactored superoxide dismutase contributes to the pathogenesis of systemic salmonellosis; Molecular Microbiology **25**(4), 785-796.

Kroll, J.S., Langford, P.R, Wilks, K.E., Keil, A.D. (1995); Bacterial Cu,Zn-superoxide dismutase: phylogenetically distinct from the eukaryotic enzyme, and not so rare after all!; Microbiology **141**, 2271-2279.

Pesce, A. et al (1997); Unique structural features of the monomeric Cu,Zn-superoxide dismutase from *Escherichia coli* revealed by X-ray crystallography; J. Mol. Biol **274**, 408-420.

Tabatabai, L.B., Pugh, G.W. (1994); Modulation of immune responses in Balb/c mice vaccinated with *Brucella abortus* Cu,Zn-superoxide dismutase synthetic peptide vaccine; Vaccine **12**(10), 919-924.

Wilks, K.E. et al. (1998); Periplasmic superoxide dismutase in meningococcal pathogenicity; Infection and Immunity, **66**., 213-217.

CLAIMS:

1. A pharmaceutical composition for vaccination, comprising:-

(i) a bacterial Cu,Zn-superoxide dismutase (Cu,Zn-SOD) of the dimeric type, or a fragment, variant or derivative of the Cu,Zn-SOD, wherein antibodies raised against said fragment, variant or derivative also bind intact full length Cu,Zn-SOD; or

(ii) a nucleic acid coding for the Cu,Zn-SOD fragment, variant or derivative; and

a pharmaceutically acceptable carrier.

2. A pharmaceutical composition according to Claim 1, wherein said composition provides protection against meningococcal infection.

3. A pharmaceutical composition according to Claim 1 or 2, wherein said composition provides protective immunity to *Actinobacillus pleuropneumoniae* infection.

4. A pharmaceutical composition according to Claims 1 or 2, wherein said composition provides protective immunity to infection from a gram negative bacterial species selected from the group consisting of *Pasteurellaceae*; *Neisseria*; *Haemophilus*; *Salmonella*; and *Escherichia*.

5. A pharmaceutical composition according to any previous claim, wherein the Cu,Zn-SOD is obtainable from a recombinant gene cloned from bacteria.

6. A vaccine comprising (i) a bacterial Cu,Zn-superoxide dismutase (Cu,Zn-SOD) of the dimeric type, or a fragment, variant or derivative of the Cu,Zn-SOD, wherein antibodies raised against said fragment, variant or derivative

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also bind intact full length Cu,Zn-SOD; or (ii) a nucleic acid coding for the bacterial Cu,Zn-SOD fragment, variant or derivative.

7. A vaccine according to Claim 6, wherein the Cu,Zn-SOD is obtainable from a recombinant gene cloned from bacteria.

8. A vaccine according to Claims 6 or 7, wherein said vaccine provides protection against meningococcal infection.

9. A method of preparing a pharmaceutical composition comprising:-

1) isolating a gene for a bacterial Cu,Zn-SOD of the dimeric type or a fragment, variant or derivative of the Cu,Zn-SOD, wherein antibodies raised against said fragment, variant or derivative also bind the full length intact Cu,Zn-SOD; and

2) (a) synthesising the Cu,Zn-SOD fragment, variant or derivative from the gene; and combining said Cu,Zn-SOD, fragment, variant or derivative, with a pharmaceutically acceptable carrier, or

(b) combining said gene with a pharmaceutically acceptable carrier.

10. A pharmaceutical preparation comprising an antibody to a bacterial Cu,Zn-SOD of the dimeric type, or a fragment, derivative or variant of the Cu,Zn-SOD, wherein antibodies raised against said fragment, derivative or variant also bind intact full length Cu,Zn-SOD; and a pharmaceutically acceptable carrier.

11. A pharmaceutical preparation according to Claim 10, wherein said antibody provides protective immunity to meningococcal disease.

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12. A pharmaceutical preparation according to Claim 10, wherein said composition provides protective immunity to *Actinobacillus pleuropneumoniae* infection.

13. A pharmaceutical preparation according to Claim 10 or 11, wherein said composition provides protective immunity to infection from a gram negative bacterial species selected from the group consisting of *Pasteurellaceae*; *Neisseria*; *Haemophilus*; *Salmonella*; and *Escherichia*.

14. A pharmaceutical preparation according to any of Claims 10 to 13, wherein said antibody displays bactericidal activity.

15. A multivalent vaccine comprising a plurality of Cu,Zn-SODs of the dimeric type, or fragments, derivatives or variants thereof, wherein antibodies raised against said fragments, derivatives or variants also bind intact full length Cu,Zn-SOD, and wherein said plurality of Cu,Zn-SODS are from the same or different species of Gram negative bacteria.

16. A multivalent vaccine comprising a bacterial Cu,Zn-SOD of the dimeric type, or fragments, derivatives or variants thereof, wherein antibodies raised against said fragments, derivatives or variants also bind intact full length Cu,Zn-SOD; and a second protein that is not a Cu,Zn-SOD.

17. A multivalent vaccine according to Claims 15 or 16, wherein said vaccine provides protective immunity to meningococcal disease.

18. Use of a bacterial Cu,Zn-superoxide dismutase of the dimeric type, or a fragment, derivative or variant of the Cu,Zn-SOD, wherein antibodies raised against said fragment, derivative or variant also bind intact full length Cu,Zn-SOD; in the manufacture of a medicament for treatment or prevention of bacterial infection.

19. Use according to Claim 18, wherein the bacterial infection is due to Gram negative species of bacteria.

20. Use according to Claims 18 or 19 wherein the bacterial infection is due to meningococcal infection.

21. Use according to Claim 18, wherein said composition provides protective immunity to *Actinobacillus pleuropneumoniae* infection.

22. Use according to Claim 19, wherein said gram negative bacterial species selected from the group consisting of *Pasteurellaceae*; *Neisseria*; *Haemophilus*; *Salmonella*; and *Escherichia*.

23. Use of a nucleic acid encoding a bacterial Cu,Zn-superoxide dismutase of the dimeric type, or a fragment, derivative or variant of the Cu,Zn-SOD, wherein antibodies raised against said fragment, derivative or variant also bind intact full length Cu,Zn-SOD, in the manufacture of a vaccine against bacterial infection.

24. Use of an antibody specific to bacterial Cu,Zn-SOD of the dimeric type, or a fragment of the antibody, in the manufacture of a medicament for treatment or prevention of bacterial infection.

25. Use according to Claim 24 wherein the antibody is a monoclonal antibody.

26. Use according to Claims 24 or 25 wherein the bacterial infection is due to meningococcal infection.

27. A method of treating or preventing bacterial infection comprising administering an effective amount of a bacterial Cu,Zn-SOD or fragment, variant or derivative of the Cu,Zn-SOD, wherein antibodies raised against said fragment, variant or derivative also bind intact full length Cu,Zn-SOD.

SEQUENCE LISTING

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Imperial College School of Medicine
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Langford, Paul Richard
Kroll, John Simon
Robinson, Andrew

<120> SUPEROXIDE DISMUTASE AS A VACCINE ANTIGEN

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20860

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Microbiological Research Authority, having a principal place of business at CAMR, Porton Down, Salisbury, Wiltshire, SP4 0JG, Great Britain, is assignee of the entire right, title, and interest for the United States of America (as defined in 35 U.S.C. §100), by reason of an Assignment to the Assignee executed on 1 MARCH 2001 of an invention known as Superoxide Dismutase as a Vaccine Antigen (Attorney Docket No._____), which is disclosed and claimed in a patent application of the same title by the inventor(s) Andrew Robinson, Andrew Richard Gorringe, John Simon Kroll, and Paul Richard Langford (said application filed on_____ at the U.S. Patent and Trademark Office, having Application Number_____).

The Assignee hereby appoints the following U.S. attorneys to prosecute this application and any continuation, divisional, continuation-in-part, or reissue application thereof, and to transact all business in the U.S. Patent and Trademark Office connected therewith: Robert Greene Sterne, Registration No. 28,912; Edward J. Kessler, Registration No. 25,688; Jorge A. Goldstein, Registration No. 29,021; Samuel L. Fox, Registration No. 30,353; David K.S. Cornwell, Registration No. 31,944; Robert W. Esmond, Registration No. 32,893; Tracy-Gene G. Durkin, Registration No. 32,831; Michele A. Cimbala, Registration No. 33,851; Michael B. Ray, Registration No. 33,997; Robert E. Sokohl, Registration No. 36,013; Eric K. Steffe, Registration No. 36,688; Michael Q. Lee, Registration No. 35,239; and Steven R. Ludwig, Registration No. 36,203. The Assignee hereby grants said attorneys the power to insert on this Power of Attorney any further identification that may be necessary or desirable in order to comply with the rules of the U.S. Patent and Trademark Office.

The Assignee hereby authorizes the U.S. attorneys named herein to accept and follow instructions from Mathys & Squire, 100 Gray's Inn Road, London WC1X 8AL, Great Britain as to any action to be taken in the U.S. Patent and Trademark Office regarding this application without direct communication between the U.S. attorneys and the Assignee. In the event of a change in the persons from whom instructions may be taken, the U.S. attorneys named herein will be so notified by the Assignee.

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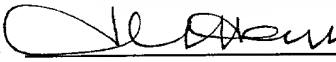
SIGNATURE: 

BY: J M HARKER

TITLE: SECRETARY MRA (CAMR)

DATE: 2 MARCH 2001

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Imperial College of Science, Technology and Medicine, having a principal place of business at Sherfield Building, Exhibition Road, London, SW7 2AZ, Great Britain, is assignee of the entire right, title, and interest for the United States of America (as defined in 35 U.S.C. §100), by reason of an Assignment to the Assignee executed on 1 MARCH 2001 of an invention known as Superoxide Dismutase as a Vaccine Antigen (Attorney Docket No. _____), which is disclosed and claimed in a patent application of the same title by the inventor(s) Andrew Robinson, Andrew Richard Gorringe, John Simon Kroll, and Paul Richard Langford (said application filed on _____ at the U.S. Patent and Trademark Office, having Application Number_____).

The Assignee hereby appoints the following U.S. attorneys to prosecute this application and any continuation, divisional, continuation-in-part, or reissue application thereof, and to transact all business in the U.S. Patent and Trademark Office connected therewith: Robert Greene Sterne, Registration No. 28,912; Edward J. Kessler, Registration No. 25,688; Jorge A. Goldstein, Registration No. 29,021; Samuel L. Fox, Registration No. 30,353; David K.S. Cornwell, Registration No. 31,944; Robert W. Esmond, Registration No. 32,893; Tracy-Gene G. Durkin, Registration No. 32,831; Michele A. Cimbala, Registration No. 33,851; Michael B. Ray, Registration No. 33,997; Robert E. Sokohl, Registration No. 36,013; Eric K. Steffe, Registration No. 36,688; Michael Q. Lee, Registration No. 35,239; and Steven R. Ludwig, Registration No. 36,203. The Assignee hereby grants said attorneys the power to insert on this Power of Attorney any further identification that may be necessary or desirable in order to comply with the rules of the U.S. Patent and Trademark Office.

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SIGNATURE: _____

BY: _____

TITLE: Head - Technology Development

DATE: 25.4.01

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Declaration for Patent Application

Docket Number: 1581.0780000

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter that is claimed and for which a patent is sought on the invention entitled Superoxide dismutase as a vaccine antigen, the specification of which is attached hereto unless the following box is checked:

☒ was filed on AUGUST 27, 1999 ;
as United States Application Number or PCT International Application Number PCT/GB99/02828 ; and
was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information that is material to patentability as defined in 37 C.F.R. § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application, which designated at least one country other than the United States listed below, and have also identified below any foreign application for patent or inventor's certificate, or PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Claimed	
9818756.0	Great Britain	27 August 1998	☒ Yes	☐ No
(Application No.)	(Country)	(Day/Month/Year Filed)		
_____	_____	_____	☐ Yes	☐ No
(Application No.)	(Country)	(Day/Month/Year Filed)		

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below.

(Application No.) (Filing Date)

(Application No.) (Filing Date)

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or under § 365(c) of any PCT international application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information that is material to patentability as defined in 37 C.F.R. § 1.56 that became available between the filing date of the prior application and the national or PCT international filing date of this application.

PCT/GB99/02828	27 August 1999	Pending
(Application No.)	(Filing Date)	(Status - patented, pending, abandoned)
(Application No.)	(Filing Date)	(Status - patented, pending, abandoned)

Appl. No. (to be assigned)
Docket No.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1 - 00

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(Supply similar information and signature for subsequent joint inventors, if any)